



**University of  
Zurich**<sup>UZH</sup>

**Zurich Open Repository and  
Archive**

University of Zurich  
University Library  
Strickhofstrasse 39  
CH-8057 Zurich  
[www.zora.uzh.ch](http://www.zora.uzh.ch)

---

Year: 2008

---

## **Cord blood cell therapy alters LV remodeling and cytokine expression but does not improve heart function after myocardial infarction in rats**

Rabald, S ; Marx, G ; Mix, B ; Stephani, C ; Kamprad, M ; Cross, M ; Boltze, J ; Briest, W ; Zimmer, H ; Deten, A

**Abstract:** **OBJECTIVE:** In this study the ability of unrestricted somatic stem cells (USSC) and mononuclear cord blood cells (MN-CBC) was tested to improve heart function and left ventricular (LV) remodeling after myocardial infarction (MI). **METHODS:** The cells were delivered by i.v. or intramyocardial injections in rat models of MI by permanent coronary artery occlusion and by ischemia/reperfusion (I/R) injury. Heart function and remodeling was followed by recurrent echocardiography over 8 or 12 weeks after which catheterization was performed. **RESULTS:** Although injected labeled cells could be observed within the myocardium for up to 6 d, there was no sign of cardiac regeneration 8 or 12 weeks after MI. However, the mRNA expression of components of the extracellular matrix was attenuated in the infarct scar 12 weeks after MI and cell injection. Additionally, the expression of interleukin (IL)-6 but not of IL-1 beta increased at the site of injury and the adjacent border-zone 12 weeks after I/R and USSC-injection. However, these effects did not translate into improved heart function or attenuated LV dilatation. **CONCLUSION:** These data indicate that cord blood cell implantation after MI acts through paracrine mechanisms to modify remodeling rather than myocyte regeneration. The role of myofibroblasts and the optimal conditions of cell application need to be determined to translate these mechanisms into functional improvement.

DOI: <https://doi.org/10.1159/000129632>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-4584>

Journal Article

Published Version



The following work is licensed under a Creative Commons: Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.

Originally published at:

Rabald, S; Marx, G; Mix, B; Stephani, C; Kamprad, M; Cross, M; Boltze, J; Briest, W; Zimmer, H; Deten, A (2008). Cord blood cell therapy alters LV remodeling and cytokine expression but does not improve heart function after myocardial infarction in rats. *Cellular Physiology and Biochemistry*, 21(5-6):395-408. DOI: <https://doi.org/10.1159/000129632>

## Cord Blood Cell Therapy Alters LV Remodeling and Cytokine Expression but does not Improve Heart Function after Myocardial Infarction in Rats

Steffen Rabald<sup>1</sup>, Grit Marx<sup>2</sup>, Brigitte Mix<sup>2</sup>, Caspar Stephani<sup>3</sup>, Manja Kamprad<sup>4</sup>, Michael Cross<sup>5</sup>, Johannes Boltze<sup>6</sup>, Wilfried Briest<sup>7</sup>, Heinz-Gerd Zimmer<sup>2</sup> and Alexander Deten<sup>8</sup>

<sup>1</sup>Department of Surgery, Leipzig University, <sup>2</sup>Carl-Ludwig-Institute of Physiology, Leipzig University, <sup>3</sup>Interdisciplinary Center for Clinical Research, Leipzig University, <sup>4</sup>Institute of Immunology and Transfusion Medicine, Max Burger Research Center, Leipzig University, <sup>5</sup>Department of Hematology/Oncology, Leipzig University, <sup>6</sup>Fraunhofer-Institute for Cell Therapy and Immunology, Leipzig, <sup>7</sup>Laboratory of Cardiovascular Sciences, National Institute on Aging, 5600 Nathan Shock Drive, Baltimore, MD 21224, USA, <sup>8</sup>Institute of Veterinary Physiology, Vetsuisse Faculty University of Zurich

### Key Words

Myocardial infarction • Heart function, remodeling • Cell therapy, animal model

### Abstract

**Objective:** In this study the ability of unrestricted somatic stem cells (USSC) and mononuclear cord blood cells (MN-CBC) was tested to improve heart function and left ventricular (LV) remodeling after myocardial infarction (MI). **Methods:** The cells were delivered by i.v. or intramyocardial injections in rat models of MI by permanent coronary artery occlusion and by ischemia/reperfusion (I/R) injury. Heart function and remodeling was followed by recurrent echocardiography over 8 or 12 weeks after which catheterization was performed. **Results:** Although injected labeled cells could be observed within the myocardium for up to 6 d, there was no sign of cardiac regeneration 8 or 12 weeks after MI. However, the mRNA expression of components of the extracellular matrix was attenuated in the infarct scar 12 weeks after MI and cell

injection. Additionally, the expression of interleukin (IL)-6 but not of IL-1 $\beta$  increased at the site of injury and the adjacent border-zone 12 weeks after I/R and USSC-injection. However, these effects did not translate into improved heart function or attenuated LV dilatation. **Conclusion:** These data indicate that cord blood cell implantation after MI acts through paracrine mechanisms to modify remodeling rather than myocyte regeneration. The role of myofibroblasts and the optimal conditions of cell application need to be determined to translate these mechanisms into functional improvement.

Copyright © 2008 S. Karger AG, Basel

### Introduction

After acute myocardial infarction (MI) had occurred, the basic pathology is characterized by irreversible and massive loss of the cardiomyocytes, which is eventually replaced by fibrous non-contractile cells to form scar tissues. Although the myocytes in the surviving myocar-

dium undergo hypertrophy this is often not sufficient to preserve heart function. As a consequence, congestive heart failure develops even though the recent advances in the therapeutic approaches, including pharmacological and interventional therapies, and cardiovascular surgery provide some improvement. As a newly developed strategy, cellular cardiomyoplasty, which involves the implantation of healthy cells into the damaged myocardium, offers the promise to replace the lost cells.

Transplantation of cells, including cardiomyocytes [1, 2], skeletal myoblasts [3], bone marrow cells [4, 5], smooth muscle cells [6], and embryonic stem cells [7] has been reported to be of potential therapeutic value for the treatment of damaged myocardium in animal models. These transplanted cells may replace infarcted myocardium and increase the number of functional cardiomyocytes, limit the scar expansion, and reduce post-infarction heart failure [8]. Also, transplanted cells may contribute to the revascularization process and enhance myocardial angiogenesis [8]. They also may serve as a platform for therapeutic gene transfer to the myocardium [9]. The beneficial effect of cell transplantation on cardiac functions in preclinical studies has led to several clinical trials [10].

Mesenchymal stem cells (MSCs) isolated from adult bone marrow have shown a great potential for cell therapy because these cells possess pluripotent capabilities [11, 12], proliferate rapidly, can 'self-renew', induce angiogenesis, and differentiate into myogenic cells [13-15]. MSCs can be isolated easily from a variety of sources, have genetic stability, and carry less immunological or ethical concerns. Hence, they were considered very suitable candidate donor cells for stem cell therapy and target cells for gene transfer [16, 17]. Recently, a pluripotent stem cell population with high proliferative potential, unrestricted somatic stem cell (USSC), was isolated from the endothelium/subendothelium layer of the human umbilical cord blood [18] that is morphologically and immunophenotypically similar to those MSCs isolated from bone marrow [19, 20]. Hence, USSCs have been suggested to be an earlier cell type than multipotent MSCs, possibly representing the precursor cells for MSCs as a comparison of the differentiation potentials of USSCs [18] and MSCs [21] has shown. In vitro and in vivo studies demonstrated that the USSCs have the potential to differentiate into osteoblasts, chondrocytes, adipocytes, neurons, and myocytes [18]. Moreover, in a sheep model, the application of USSCs did not induce detectable tumors in a long-term study after transplantation [18]. Therefore, USSCs could be highly promising precursor cells

for cardiac implantation after a myocardial infarction.

This study was performed to test the ability of USSCs to improve heart function and left ventricular (LV) remodeling after MI. The cells were delivered by intramyocardial injections in rat models of MI by permanent coronary artery occlusion and by ischemia/reperfusion (I/R) injury. Additionally, mononuclear cord blood (MN-CBCs) cells without further separation were tested and the effects compared.

## Materials and Methods

### *Animal model*

Myocardial infarction was induced in male (3.5 months of age and 291±4 g of body weight at the beginning of the study) spontaneously hypertensive rats (SHR, Charles River) and female (3 month of age and 242±14 g of body weight at the beginning of the study) Sprague-Dawley rats (SD, Charles River) by ligation of the left anterior descending coronary artery (LAD) as previously described [22]. For the I/R experiments which were done only in the SD rats, a small ring (cord diameter 1 mm) was placed under the ligature. After 60 min of occlusion, the rats were again anesthetized, the chest was opened and both, the ring and the ligature were removed. Successful reperfusion was verified by Evans blue infusion in pilot experiments (not shown), but became also momentarily overt by the color change of the previously ischemic myocardium from pearl grey to pink.

After 24 h the surviving SHR (n=46) were randomly selected to receive either cells (MI+MN-CBC, 3×10<sup>6</sup> cells in 500 µl medium, n=23) or medium (MI-CTRL, n=23) via a tail vein. The surviving SD rats (n=47) were also randomly selected after 24 h for direct intramyocardial injection of either cells (each 1×10<sup>6</sup> cells, n=14 for MN-CBCs and n=15 for USSCs) or medium (n=18). In the I/R experiments, the rats were randomly selected to either receive cells (1×10<sup>6</sup> USSCs, n=9) or medium (n=9) by direct intramyocardial injection at the time of reperfusion (Tab. 1). The intramyocardial injections were performed at two sites (10 µl each) at the mid-anterior and mid-lateral left ventricular wall using a precision syringe (22G, Hamilton), the needle was equipped with additional tubing as retardant. All rats in the I/R sub-study received additional immune-suppressive therapy with Cyclosporine (Sandimmun Neoral®, Novartis, Germany, 15 mg/kg/d) beginning the day before surgery.

Twelve additional SHR were used to track injected CFSE-labeled cells after 6 and 24 h (n=4 for MN-CBCs and n=2 for medium at each time-point). Also 16 additional SD rats were analyzed for injected labeled cells immediately (MI, n=4), 1 d (MI, n=3) and 6 d (each n=3 for both, MI and I/R) after injection. Medium-treated MI hearts at 1 d after injection served as controls (n=3).

Sham-operated animals (n=24 for SHR and n=18 for SD) underwent the same procedure except that no ligation was performed. Also the groups of sham operated animals were divided into sub-groups to receive either cells or medium. These groups were combined for subsequent comparison with MI

**Table 1.** Study design and animal numbers. Total numbers of animals and in each group; in brackets: survived the first day after surgery; in parenthesis: survived observation period; MI: myocardial infarction; I/R ischemia/reperfusion; CTRL: injected with medium; MN-CBCs: injected with human mononuclear cord blood cells; USSCs: injected with human unrestricted somatic stem cells.

	SHR (84)	SD (104)	
	MI (8 w)	MI (12 w)	I/R (12 w)
sham	24	18	
all MI (I/R) [at day 1]	60 [46]	68 [47]	18 [18]
- CTRL	23 (14)	18 (18)	9 (9)
+ MN-CBCs	23 (13)	14 (11)	
+ USSCs		15 (14)	9 (9)

(I/R) rats, since there were no statistically significant differences between the differently treated sham groups. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the appropriate State agency of Saxony.

#### *Isolation of human cord blood cells*

Human cord blood was collected from umbilical cord vein of full-term pregnancies, after informed consent. The investigation conforms with the principles outlined in the Declaration of Helsinki. Mononuclear cord blood cells (MN-CBCs) were isolated by Ficoll-Hypaque density gradient separation followed by erythrocyte lysing in distilled water. MN-CBCs were cryopreserved in 8% dimethyl sulphoxide (DMSO) and 92% fetal calf serum (FCS) at  $-196^{\circ}\text{C}$  (liquid nitrogen). After thawing by stepwise dilution in RPMI 1640 (PAA Laboratories) and washing by DNase-containing buffer (DNase I, Roche), the cells were analyzed by flow cytometry and used for transplantation. To isolate unrestricted somatic stem cells (USSCs), the mononuclear cell fraction was isolated by Ficoll-Hypaque density gradient separation and plated at  $4\text{--}6 \times 10^6$  cells/ml and incubated at 1% O<sub>2</sub> in cell culture flasks (Greiner Bioscience). Medium contained 70 % low glucose (5mM) DMEM (Sigma), 30 % FCS (Pan Biotech) and was changed every 7 days until formation of colonies of adherent cells was observed as previously described [18]. Cells were harvested after reaching confluency and further expanded with a lower concentration of Dexamethason at a density of  $4 \times 10^4$  cells/cm<sup>2</sup>. Only USSCs from passage 5-7 were used for experiments.

#### *Flow Cytometry*

MN-CBCs were analyzed for the cell surface antigens CD45, CD34, CD133 and the homing receptor CXCR4 compared to isotype controls in order to estimate the relative number of stem cell/progenitor population. CD45-PC7 and CD34-APC antibodies were purchased from Beckman Coulter (Immunotech) while the CD133-PE antibody was obtained from Miltenyi Biotech. The CXCR4 antibody was purchased from R&D Systems and the secondary goat-anti-mouse-FITC antibody from DAKO. Cells were incubated with antibodies 30 minutes at  $4^{\circ}\text{C}$  and washed twice. After fixation in 3% formaldehyde the cells were analyzed using FACSCalibur and CellquestPro software (Becton Dickinson) according to the manufactures protocol. The gating strategy for the hematopoietic stem cells was according to the ISHAGE guidelines [23].

USSCs were analyzed for the cell surface antigens CD13, CD34, CD90 (all Becton Dickinson), and CD105 (Serotec) compared to the isotype control. Cells were incubated with anti-

bodies for 40 minutes at  $4^{\circ}\text{C}$  and washed once with PBS. The cells were resuspended in PBS and immediately analyzed using FACSCalibur and CellquestPro software (Becton Dickinson).

#### *Cell labeling*

For a simple and rapid recovery the cells were labeled with  $5 \mu\text{M}$  5,6-Carboxyfluoresceindiacetat-succinimidylester (CFSE, Molecular Probes). The cells were incubated in RPMI with 1% CFSE for 10 minutes at  $37^{\circ}\text{C}$  and then washed twice with RPMI and 10% FCS. Cells were resuspended in RPMI 1640 / 1% FCS, analyzed by flow cytometry and used for transplantation.

#### *Echocardiography*

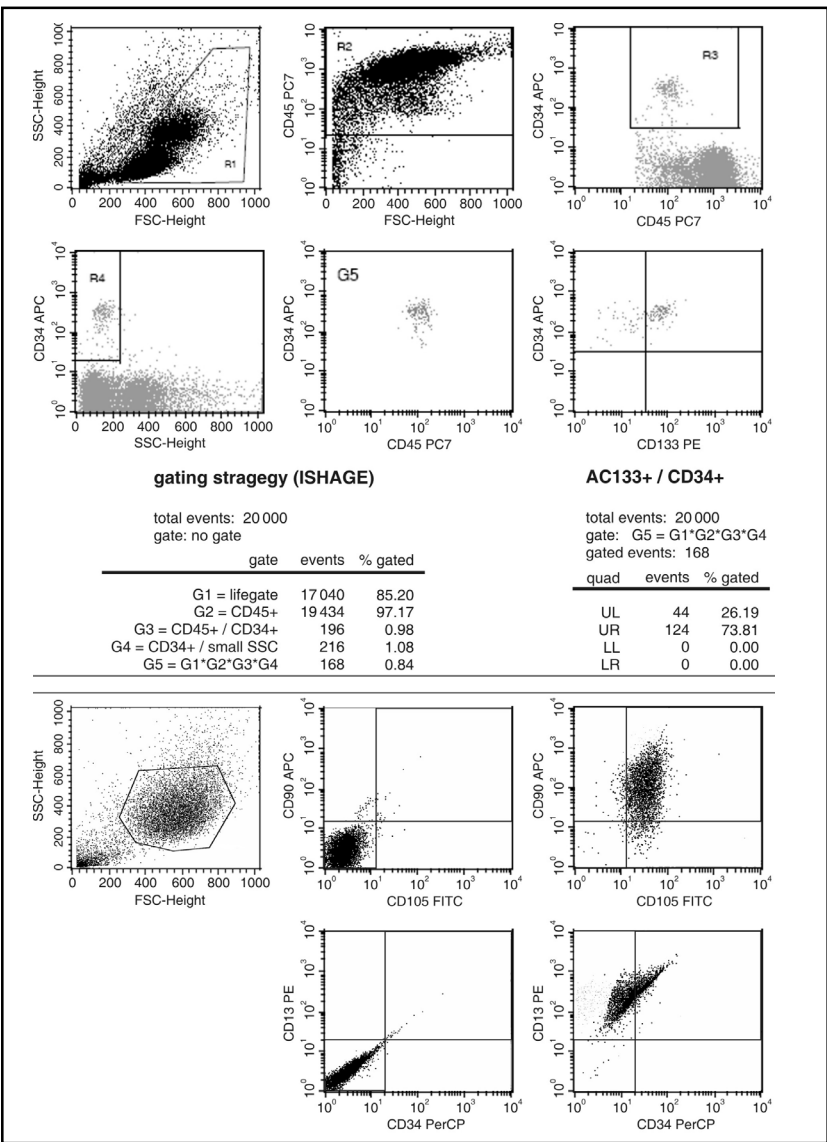
Echocardiographic measurements were performed with a commercially available ultrasound system (GE Vivid 7 equipped with an 11.5 MHz sector scan probe, GE Healthcare). Examinations were performed in spontaneously breathing animals, under 1.5 % isofluran anesthesia, in left lateral decubitus position. Parasternal short axis view was recorded at the largest round diameter of the left ventricle. For recording of the contrast enhanced loops, a tail vein was punctured, and contrast medium (Optison®, GE Healthcare) was injected after adjusting the apical view and reducing the ultrasound beam power. Criteria for all apical views were to display the mitral valve opening and the LV in its longest cross section with minimal displacement effects on wall thickness. Within these criteria the four chamber view was identified as the view with the largest cross-section of the right ventricle (RV). The two chamber view was taken pragmatically orthogonal from the four chamber view. Additionally, a standard record of established Doppler measurements was taken.

Offline analyses from the digital raw data were performed on a PC using ultrasound analysis software (EchoPac PC, GE Healthcare). All measurements were done in 3 to 5 consecutive heartbeats. Left ventricular end systolic (LVESV) and end diastolic (LVEDV) volume were measured by the software's internal algorithm (SIMPSON, biplane) from the paired apical views. Ejection fraction (EF) was calculated by  $\text{EF} = (\text{LVEDV} - \text{LVESV}) / \text{LVEDV}$ . From short axis view, left ventricular end systolic (LVESA) and end diastolic (LVEDA) area were measured. Fractional area change (FAC) was calculated by  $\text{FAC} = (\text{LVEDA} - \text{LVESA}) / \text{LVEDA}$ .

#### *Hemodynamic measurements*

At the end of the observation periods, left and right heart function was measured in closed-chest spontaneously breathing rats anesthetized with thiopental sodium (Trapanal® 80

**Fig. 1.** FACS analysis of MN-CBCs for CD45, CD34, and CD133 (upper panels) and of USSCs for CD90, CD105, CD13, and CD34 (lower panels with isotype controls in the middle column) isolated from human cord blood. The gating strategy was according to the ISHAGE guidelines.



mg/kg i.p., Byk Gulden) using ultraminiature catheter pressure-transducers (3F, Millar Instruments Inc.) [22]. Cardiac output was measured by the thermodilution method (Cardiomax-IIR, Columbus Instruments) [24].

#### Tissue collection and infarct size measurement

After the hemodynamic measurements had been obtained, the hearts were arrested in diastole by KCl injection and rapidly excised. The hearts from the SHR sub-study were fixed in 4% paraformaldehyde, cut into 5 transversal sections and photographed. The MI size was calculated as ratio of the infarcted segment to the total LV perimeter averaged between endocardial and epicardial measurements using the ImageJ 1.33k software (NIH), and the average MI was expressed as a percentage of total LV perimeter [25, 26]. Additionally, segments at mid-papillary level were paraffin-embedded, and sectioned at 8  $\mu$ m. The hearts from the SD rats were transversally cut in two halves approximately at mid-papillary level (site of largest infarct extension). The apical parts were used for histological analyses. From the basal part, the RV was trimmed away

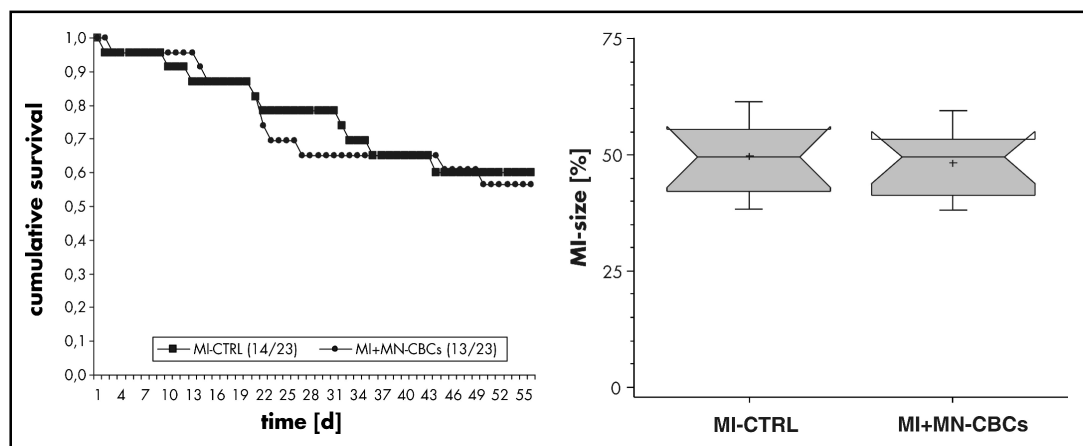
and the infarct scar was excised as well as a 2-3 mm border zone adjacent to the infarct scar. All tissue pieces were snap-frozen in liquid nitrogen for later analyses.

In additional experiments the hearts were harvested immediately as well as 6 h, 24 h and 6 d after injection of labeled cells or medium. The hearts were embedded in O.T.C. compound, cryopreserved in melting methylbutan, and subsequently sectioned at 7  $\mu$ m. After DAPI-staining of the nuclei the sections were directly analyzed under a fluorescence-microscope (Zeiss Axioskop) and photographed.

#### Immunohistochemical analysis

The following antibodies were used: anti-smooth muscle actin (SMA; mouse monoclonal, dilution 1:1.000; Boehringer Mannheim), and desmin (rabbit polyclonal, dilution 1:500; PharMingen). Immunohistochemical analysis was performed according to the recommendations supplied by the manufacturer. Sections known to stain positively were included in each batch and negative controls were performed by replacing the primary antibody with serum (Sigma-Aldrich).

**Fig. 2.** Cumulative survival (left) and infarct-size (right) 8 weeks after myocardial infarction (MI) and medium (CTRL) or mononuclear cord blood cell injection (MN-CBC).



#### *RNase Protection Assay (RPA)*

Total RNA isolation and RNase protection assay (RPA) were performed as previously described [22, 27].

#### *Statistical Analysis*

The data are expressed as mean (SD). A Kruskal-Wallis ANOVA on ranks was used for multigroup comparison subsequently utilizing multiple comparison procedure according to Dunn's method (SigmaStat 3.10, SPSS Corp.). Cumulative survival was analyzed by Kaplan-Meier-Plot including overall comparison according to Mantel-Cox, Breslow, and Tarone-Ware (SPSS 13.0 for Windows, SPSS Corp.) A value of  $p < 0.05$  was considered statistically significant.

## Results

#### *Cell characterization*

Nearly all of the alive, isolated mononuclear cord blood cells were positive for CD45. Only  $0.7 \pm 0.09$  % of those (medium intense CD45+) cells were also positive for CD34, but  $79.4 \pm 2.2$  % of the CD45+/CD34+ cells stained for CD133 (Fig. 1). Moreover, after cryopreservation  $30.6 \pm 16.2$  % of the CD34+ cells also expressed the stem cell receptor CXCR4. Nearly all of the cultured USSCs were positive for CD105 and CD13 surface antigens and, also for CD90. However, staining for CD34 was weak (Fig. 1).

#### *Survival and infarct size*

The mortality within the first 24 h after coronary artery occlusion in the SHR sub-study was 19% (14/72). The animals which had survived for 24 h, were randomly selected to receive either MN-CBCs or medium. Twelve rats were used to track the injected cells after 6 and 24 h while the remaining 46 rats were followed for 8 weeks.

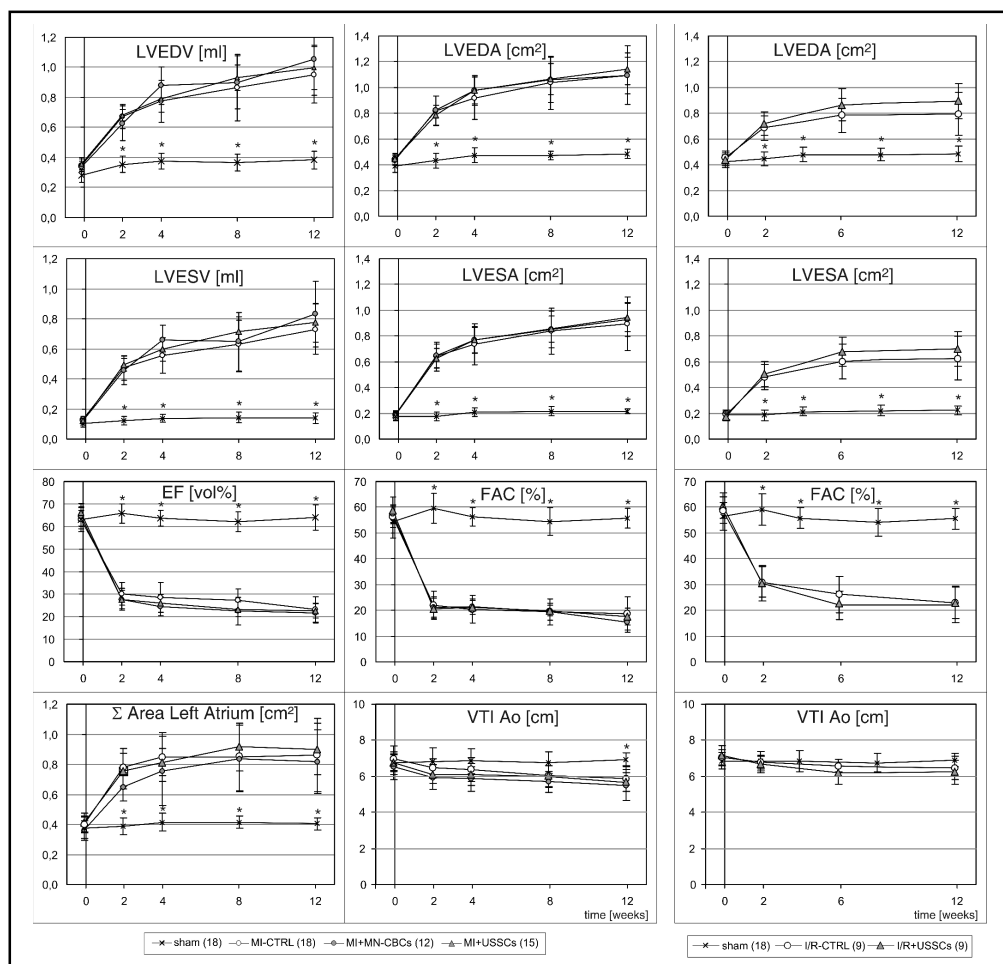
During this observation period the cumulative survival of the MI+MN-CBC group (13/23) was not significantly different from that of the medium-treated MI-CTRL group (14/23) (Fig. 2, left). There was also no difference in the infarct size (Fig. 2, right). None of the sham-operated rats died.

The mortality within the first 24 h after coronary artery occlusion in the SD MI sub-study was 27% (21/78). The surviving animals were also randomly selected to receive either cells or medium. Ten rats were used to track the injected cells while the remaining 47 rats were followed for 12 weeks (Tab. 1). During this observation period 2 of the MN-CBC-treated, but none of the medium-treated rats died. Two more rats (each 1 of the MN-CBC-treated and 1 of the USSC-treated rats) died during the night after last echocardiography, but before final hemodynamic measurements. Statistics of cumulative survival was not done due to the small numbers. Also, MI size was not measured, since a part of the hearts was used for molecular biological analyses. None of the I/R rats or of the sham-operated rats died.

#### *Echocardiography*

Echocardiography was performed in the SD sub-studies only. Successful induction of MI was confirmed by an echocardiographically akinetic LV free wall in all animals a few hours after surgery (details not shown). Severely impaired heart function became overt by greatly reduced EF (and FAC) 2 weeks after MI (Fig. 3). Thereafter, it only slightly decreased over time. LV dimensions at both, end-diastole and end-systole, increased dramatically 2 weeks after coronary artery occlusion. Thereafter, they further increased, but less pronouncedly. Reduced LV pump function was also indicated by an

**Fig. 3.** Summary of echocardiographic data over 12 weeks after permanent coronary artery occlusion (MI, left and middle column) or ischemia/reperfusion (I/R, right column). LVED(S)V(A) left ventricular end-diastolic (-systolic) volume (area); EF ejection fraction; FAC fractional area change; VTI velocity time integral. Data are mean $\pm$ SD; \* $p$ <0.05 for all MI-groups vs sham (data for 6 weeks I/R were calculated vs the mean of 4 and 8 weeks sham).



increased size of the LA. VTIAo, on the other hand, decreased only slightly and this decrease was statistically significantly different from sham-operated rats 12 weeks after surgery (Fig. 3). Most importantly, these changes were observed in all MI rats, the cell-injected rats being indistinguishable from the medium-injected MI-CTRLs.

FAC decreased severely 2 weeks after I/R and further deteriorated over time thereafter (Fig. 3, right column). Moreover, LV dimensions increased 2 weeks after I/R, but this increase was less pronounced compared to MI. VTIAo was not statistically significantly reduced after I/R. Importantly again, these changes were not different between USSC-injected and medium-injected I/R rats.

### Hemodynamic measurements

Heart function could not be measured in all rats, since in all MI-groups 2 rats died shortly after thiopental injection for anesthesia. Hemodynamic data revealed severely depressed heart function after MI. The LV systolic pressure (LVSP), LVdP/dtmax and LVdP/dtmin severely decreased after MI while LV end-diastolic pres-

sure (LVEDP) and  $\tau$  increased (Tab. 2). Total peripheral resistance (TPR) also declined, but mainly due to the reduced mean arterial pressure, since cardiac output decreased significantly only in the SHR after MI. Impaired pump function of the LV became also overt by increased parameters of the RV. Importantly, all these parameters of deteriorated LV and elevated RV function were not significantly different between medium-injected and cell-injected MI-rats, except for TPR in the SD MI-sub-study.

The hemodynamic consequences were less profound 12 weeks after I/R (Tab. 2). LVSP remained normal while LVEDP increased only slightly. Consequently, RV function was comparable to sham-operated rats. The parameters of LV contractility and relaxation, on the other hand, were significantly impaired after I/R. This, however, was not different between USSC- and medium-injected rats.

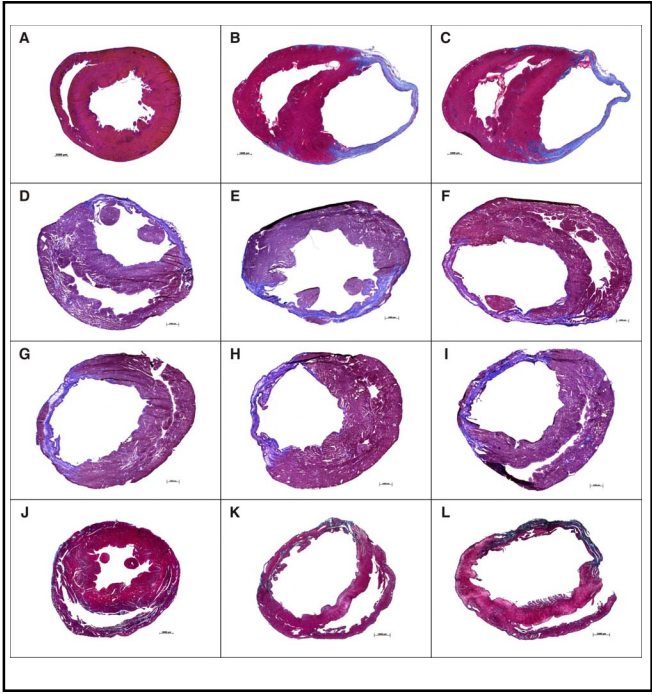
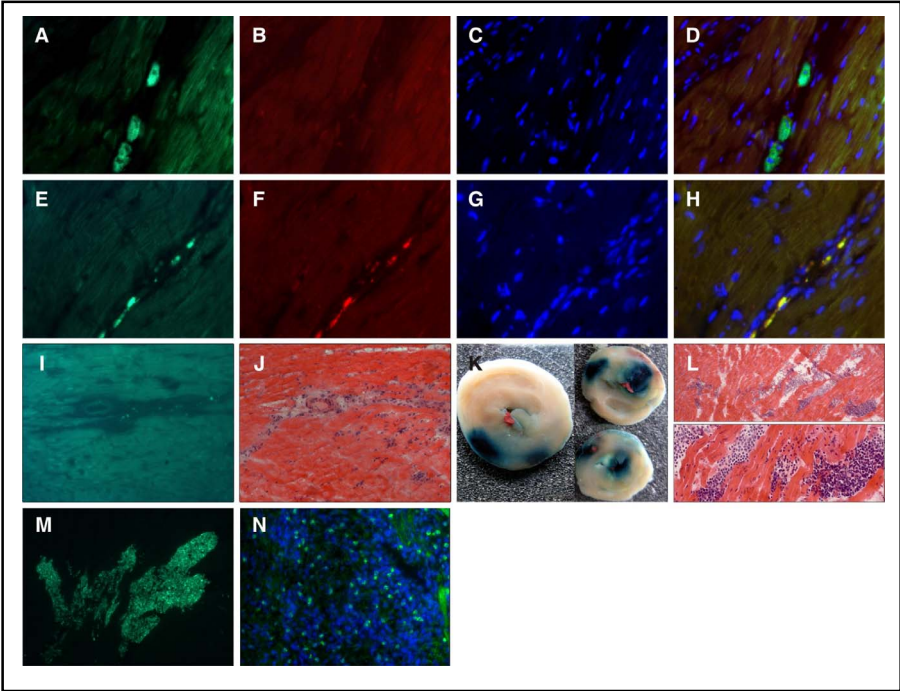
### Cell tracking

CFSE-labeled cells were detected by green fluorescence (Fig. 4). They were observed after i.v. injection in paravascular regions of the border zone adjacent to



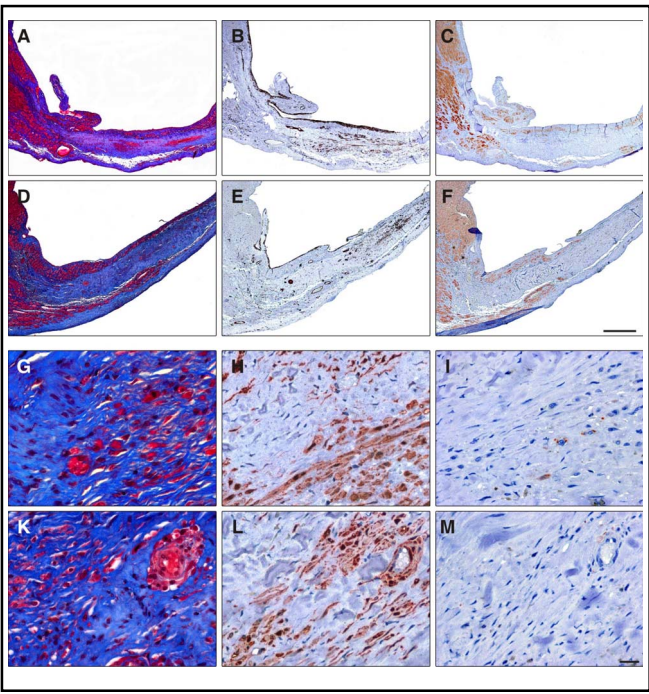


**Fig. 4.** Tracking of CFSE-labeled cells. Green, red, blue fluorescence, and merge in the border-zone 48(24) h after MI and i.v. cell (A-D) or medium (E-F) injection. I and J Green fluorescence and HE staining of a consecutive section from the border-zone 48(24) h after MI and i.v. cell injection. K Amidoblack-injections to verify intramyocardial delivery. HE staining and green fluorescence immediately (L and M) or 6 d (N) after I/R and intramyocardial injections.



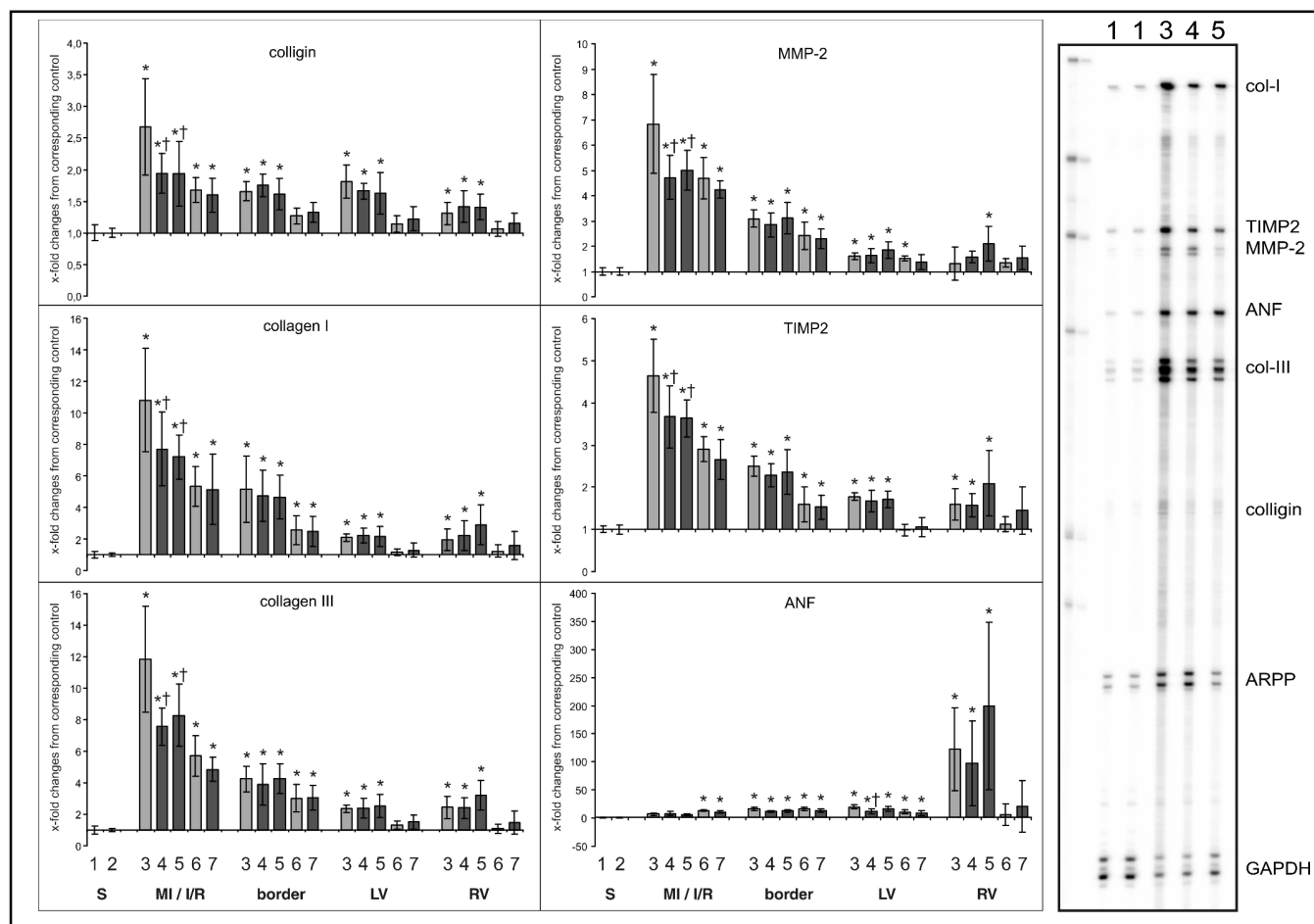
**Fig. 5.** Histological overviews (Mason's trichrome) after MI and I/R. A SHR sham; B SHR MI-CTRL; C MI+MN-CBCs; D/G SD MI-CTRL; E/H SD MI-MC-CBCs; F/I SD MI+USSCs; J SD sham; K SD I/R-CTRL; L SD I/R+USSCs.

the infarct area (identified as infiltration zone in HE stained serial sections) 6 and 24 h after cell injection (Fig. 4A-J). Green spots were also detected in medium-injected MI-



**Fig. 6.** Overviews (2 upper rows) and higher magnification (2 lower rows) of Mason's trichrome (left) and consecutive anti-SMA (middle) and anti-desmin (right) staining 12 weeks after MI and medium (A-C and G-I) or MN-CBC (D-F and K-M) injection.

CTRL-hearts (Fig. 4E-H). However, these spots showed also red fluorescence and could not be assigned to a nucleus. Therefore, they represent non-specific auto-fluo-



**Fig. 7.** Summary of mRNA expression of extracellular matrix components (left) and representative RPA (right). 1 sham LV; 2 sham RV; 3 MI-CTRL; 4 MI+MN-CBCs; 5 MI+USSCs; 6 I/R-CTRL; 7 I/R+USSCs. Data are normalized to the acidic ribosomal phosphoprotein (ARPP) and shown as mean $\pm$ SD. \*  $p < 0.05$  vs corresponding sham; †  $p < 0.05$  vs MI-CTRL.

rescence. Successful intramyocardial cell injection was verified immediately after injection (Fig. 4K-M). Also 6 d after injection, labeled cells could be detected in the hearts, but apparently in smaller numbers (Fig. 4N).

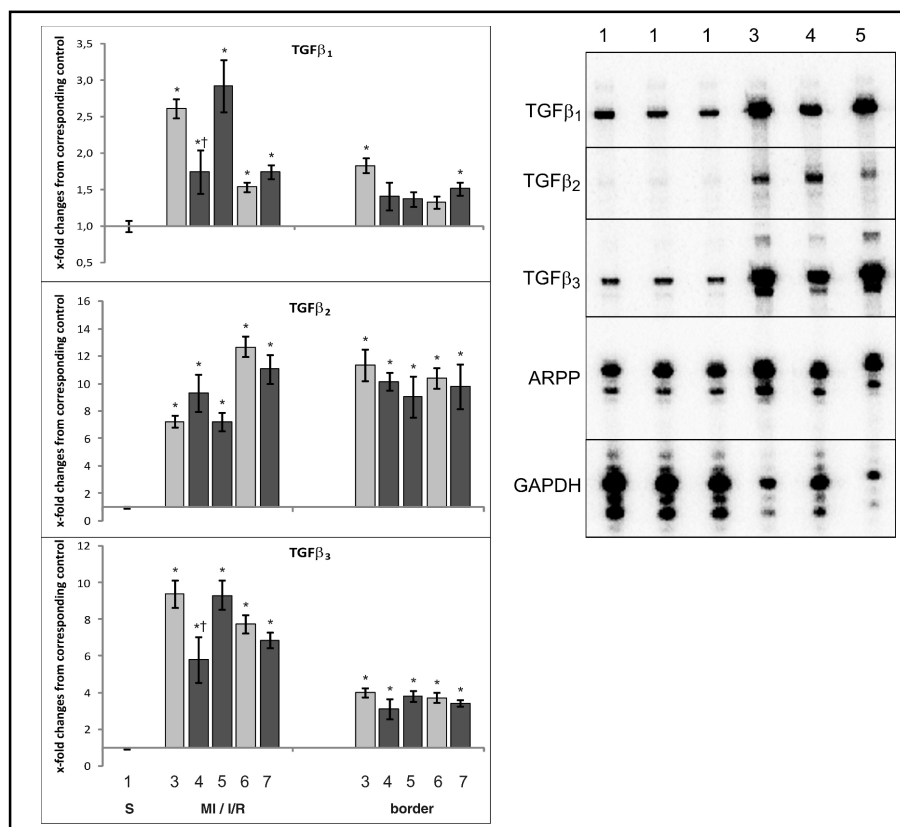
#### Histological and Immunohistochemical analyses

All sections of the MI-hearts revealed typical changes for old infarcts with a subendocardial layer as well as patchy subepicardial amounts of surviving myocytes (Fig. 5). Despite similar infarct-sizes and no obvious signs of regeneration at the site of former injury, Mason's trichrome overview staining showed islets of red-stained cell bodies within the blue-stained collagenous scar (Fig. 6, left column). Immunohistochemical analysis revealed that those cells were positive for  $\alpha$ -SMA but negative for desmin (Fig. 6, middle and right column). Moreover, neither occurrence nor characteristics of those cell islets were different between cell-injected and medium-injected hearts.

#### Expression of components of the extracellular matrix and of cytokines

The mRNA-expression of some major components of the extracellular matrix (ECM) namely collagen I, collagen III, the collagen chaperon colligin (also known as Hsp47), matrix-metalloproteinase (MMP)-2 and tissue inhibitor of matrix-metalloproteinases (TIMP) 2 substantially increased after MI (Fig. 7). This increase was most pronounced in the infarct area, but was also observed in the border-zone adjacent to the MI as well as in the non-infarcted LV and RV. Interestingly, these changes were significantly attenuated in both, the MN-CBC and the USSC-treated hearts. However, this was observed only in the infarct area in which the ECM expression in the cell-treated hearts after MI was comparable to that after I/R. In general, ECM expression also increased after I/R, but to a lesser extent and only in the area of injury and in the adjacent border-zone. However, the increase in ECM expression 12 weeks after I/R was not different

**Fig. 8.** Summary of mRNA expression of TGF- $\beta$ -isoforms (left) and representative RPA (right). 1 sham LV; 3 MI-CTRL; 4 MI+MN-CBCs; 5 MI+USSCs; 6 I/R-CTRL; 7 I/R+USSCs. Data are normalized to the acidic ribosomal phosphoprotein (ARPP) and shown as mean $\pm$ SD; \*  $p < 0.05$  vs corresponding sham; †  $p < 0.05$  vs MI-CTRL.



between the USSC-treated and the medium-treated hearts.

Also ANF expression was induced after MI and I/R (Fig. 7). The most pronounced increase was observed after MI in the RV, in which it was absent after I/R. However, ANF expression was not influenced by cell-treatment, except for a slight but statistically significant reduction in the non-infarcted LV of the MN-CBS-treated rats.

The mRNA-expression of the TGF- $\beta$  isoforms was induced after MI (Fig. 8). TGF- $\beta_1$  expression increased predominantly in the infarct area after permanent coronary artery occlusion. This increase was attenuated in the rats treated with MN-CBCs, but not in the rats treated with USSCs. The induction in TGF- $\beta_3$  was similar to that of TGF- $\beta_1$ , but generally more marked. Again, the most pronounced increase occurred in the infarct area after permanent coronary artery occlusion and was attenuated in the MN-CBC treated rats, but not after treatment with USSCs. Also TGF- $\beta_2$  mRNA expression was induced after injury. In contrast to the other isoforms, the increase in TGF- $\beta_2$  was not reduced after MN-CBC therapy but slightly more pronounced, although this did not reach statistical significance. Moreover, the induction of TGF- $\beta_2$  was more pronounced after I/R and in the border zone

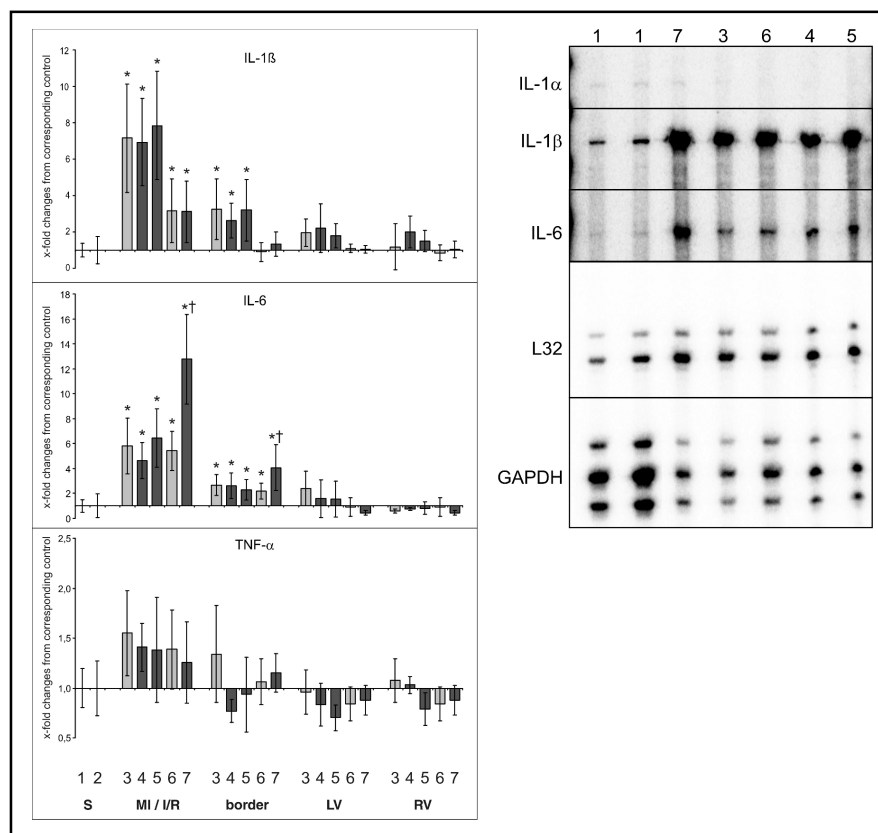
adjacent to the infarct area after permanent coronary artery occlusion (Fig. 8).

The mRNA-expression of interleukin (IL)-1 $\beta$  and IL-6, but not of tumor necrosis factor- $\alpha$  was induced in the area of injury and in the adjacent border-zone, but not in the non-infarcted LV and RV (Fig. 9). Moreover, the increase in IL-1 $\beta$  was more pronounced after MI compared to I/R, but not influenced by cell application. In contrast, the increase in IL-6 expression was comparable after MI and I/R. Moreover, it was significantly pronounced in both, the injured area and the adjacent border-zone of the USSC-treated hearts. However, this was observed after I/R but not MI.

## Discussion

After intramyocardial injection of both, MN-CBCs and USSCs, into the site of injury 24 h after permanent coronary artery occlusion, the mRNA expression of components of the ECM was attenuated in the scar after 12 weeks (Fig. 7). This was accompanied by a reduced induction of TGF- $\beta_1$  and TGF- $\beta_3$  expression after MN-CBC, but not after USSC treatment (Fig. 8). Additionally, the expression of IL-6 but not of IL-1 $\beta$  was increased

**Fig. 9.** Summary of mRNA expression of cytokines (left) and representative RPA (right). 1 sham LV; 2 sham RV; 3 MI-CTRL; 4 MI+MN-CBCs; 5 MI+USSCs; 6 I/R-CTRL; 7 I/R+USSCs. Data are normalized to the mRNA of the ribosomal protein L32 and shown as mean $\pm$ SD; \*  $p < 0.05$  vs corresponding sham; †  $p < 0.05$  vs I/R-CTRL.



at the site of injury and the adjacent border-zone 12 weeks after I/R and USSC-injection (Fig. 9). However, these effects did not translate into improved heart function or attenuated LV dilatation (Tab. 2 and Fig. 3). Moreover, there was no sign of regeneration (Fig. 5 and 6).

The effect of stem and progenitor cells after MI is still controversial. In this study, USSCs as precursors of MSCs with remarkable pluripotent capabilities [18, 28] and, therefore, as promising candidates were compared with MN-CBCs in regard to their effectiveness. The latter have recently been shown to exert beneficial functional effects in a very similar setup of experimental stroke in SHR [29, 30]. The lack of any obvious myocardial regeneration is in line with previous reports showing that myocyte transdifferentiation of various progenitor cells originating from the bone marrow (BM) is a very rare event, if any [31-35]. Moreover, intracoronary injection of USSCs 7 d after I/R in a porcine model did not attenuate MI-induced LV remodeling or ameliorate global and regional LV dysfunction [36]. An earlier study, however, reported that direct injection of USSCs into the border zone of the infarct area 4 weeks post-MI in a porcine model of permanent occlusion resulted in significant improvement of ejection fraction compared to medium-treated controls at 8 weeks post-MI [37]. Also in rats

direct injection of MN-CBCs at 1 week after permanent coronary artery occlusion significantly improved LV function 4 weeks after treatment [38]. Additionally, intravenous injection of MN-CBCs 1 day after permanent coronary artery ligation in mice was reported to reduce infarct size [39].

It is, however, difficult to reconcile the data of the present study with the findings of the previous reports. A number of factors like pre-selection and pre-treatment of the cells, time and route of application, isolation and storage method, and the experimental setup might be of influence. The timing of cell injection cannot explain the observed lack of benefit on LV remodeling, function or infarct size, since previous studies showed beneficial effects of cord blood cell injection as early as 20 min, 60 min, or 1 day [39-41] after coronary ligation, but also as late as 1 or 4 weeks after ligation [37, 38]. The results of these previous studies were observed 3-4 weeks after MI, also indicating that the 8-12 week follow-up period was sufficient to allow detection of an effect of cell transplantation.

Cord blood cells are assumed to possess immune-privileges and are supposed to be hypo-immunogenic [42]. Furthermore, in a previous study labeled USSCs were detected in the infarct zone 4 days after injection with

and without immunosuppression, suggesting the absence of hyper-acute rejection [36]. Since hypoimmunogenicity of the umbilical cord blood derived cells is still questionable and, also, may change in case differentiation takes place, an additional immunosuppressive therapy was applied in the I/R sub-study to exclude the possibility that the lack of functional effects was due to rejection.

For the experiments in hypertensive rats, the cells were injected intravenously after permanent coronary occlusion. The low level of engraftment of these cells into the injured adult heart after tail vein infusion may not be surprising, because the occluded artery makes it difficult for the injected cells to reach the infarcted myocardium. This problem is compounded by the fact that intravenously injected cells are likely to be sequestered in various organs. However, labeled cells were clearly detected in paravascular regions of the border zone adjacent to the infarct area (Fig. 4), but not quantified. A much higher number of cells was detected after direct cell injection, but considerably decreased after 6 d (Fig. 4). Only freshly cultured USSCs were used in this study, but cryopreserved MN-CBCs. Although a small portion of the cryopreserved cells is not viable after thawing, the majority of these cells can unrestrainedly be cultured (details not shown). To generally minimize cell damage during injection, a canula with a large diameter was used (22G). It can also not completely be ruled out that CFSE labeling impairs the capabilities of the cells. In pilot studies, however, it did not affect proliferation and viability of cultured MN-CBCs (details not shown). Nonetheless, unlabeled cells were used for the long-term experiments, since after that time also other factors like fusion or loss of CFSE during proliferation might complicate possible interpretations. Since the histological examination revealed typical changes for old infarcts and, more importantly, the cell-treated hearts were indistinguishable from the medium-treated controls, further staining for typical cardiac transcription factors (GATA4, MEF2) was not performed. Therefore, it can not completely be ruled out that a very small number of the injected cells could be able to differentiate and remained at the site of injury. This would be in line with a previous study in pigs reporting only a few of the injected USSCs after 4 weeks [36]. Moreover, the injected USSCs did not transdifferentiate into a cardiomyocyte or endothelial phenotype since they were negative for Troponin and vWF, but were still CD45 positive.

It has also been hypothesized that enhanced neoangiogenesis after BM cell application might contribute to improved ventricular function after MI. From re-

cent studies, however, involvement of BM derived cells in peri-infarct or hypoxia-induced angiogenesis has been questioned [34, 43], although this is very likely for endothelial progenitor cells. Neoangiogenesis in scar tissue might be important for remodeling, but was not analyzed in this study, since myocardial functional improvement directly caused by enhanced neoangiogenesis cannot be expected, given the absence of contractile cells in the scar.

On the other hand, paracrine mechanisms have been suggested to contribute to the beneficial effects of progenitor cell application after MI [44, 45]. Interestingly, the application of both, MN-CBS as well as of USSCs after MI attenuated the expression of ECM components (Fig. 7) pointing to such indirect mechanisms even in the absence of regeneration and even 12 weeks after the injury. Notably, this was observed in the infarct scar only and only after permanent coronary artery occlusion. Moreover, the induction of ECM expression after MI was attenuated to levels comparable to that after I/R, but did not translate into functional improvement. This might designate the critical importance of the time of cell injection after injury. Since labeled injected cells were detected at 6 d after I/R (Fig. 4), it might be speculated that the substantial differences in the pathomechanisms after permanent occlusion and I/R may also require different strategies for cell therapy. The fact that USSCs administered after I/R led to increased expression of IL-6, but not of IL-1 $\beta$  in the infarct and the peri-infarct (Fig. 9), further suggests paracrine effects after cell therapy.

It has now been accepted that the infarct scar is a highly dynamic tissue for months and even years after MI in rodents and men [27, 46, 47]. Myofibroblasts are the main mediators of fibrogenesis and remodeling after myocardial injury [48], but may also serve as precursor cells necessary for angiogenesis [49] and, therefore, are essential for the capacity of the infarcted heart to heal. Recently, a high number of eGFP positive fibroblasts and myofibroblasts were observed in the infarct and peri-infarct in a mouse model of MI after BM replacement by eGFP expressing cells. This suggests that BM derived myofibroblasts may be of particular importance for the benefit of BM cells in myocardial healing processes [34]. A recent report, on the other hand, did not show differentiation of BM derived cells into myofibroblasts [50]. Myofibroblast-like cells were observed in the infarct area of both, cell and medium treated MI hearts (Fig. 6), but not quantified. Since the injected cells were not labeled and also screening for human chromosome by FISH was not done, it remains to be elucidated, if injected cells di-

rectly take part in remodeling by transformation into myofibroblasts or rather indirectly modulate the remodeling by paracrine mechanisms like the expression of cytokines and growth factors. It should be pointed out that rats injected with inactive cells after MI would be the more appropriate controls to test for released factors or the induction of active processes within the injured tissue. However, the data on TGF- $\beta$  expression indicate differential effects induced by MN-CBCs and USSCs (Fig. 8). This also indicates that additional factors different from TGF- $\beta$  seem to be important for cardiac remodeling after MI.

In summary, this study indicates that cord blood cell implantation after myocardial infarction acts through paracrine mechanisms to modify remodeling rather than

myocyte regeneration. The role of myofibroblasts and the optimal conditions of cell application need to be determined to translate these mechanisms into functional improvement.

## Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (ZI 199/10-3 and ZI 199/10-4) and by grants of the Medical Faculty of the University of Leipzig (formel.1-19), of the Federal Ministry of Education and Research (BMBF, NBL-3-Förderung; Kennzeichen 01ZZ0106) and of the Novartis-Foundation (Nürnberg, Germany, Graduiertenstipendium 2006 to AD).

## References

- Leor J, Patterson M, Quinones MJ, Kedes LH, Kloner RA: Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation* 1996;94:II332-336.
- Watanabe E, Smith DM, Delcarpio JB, Sun J, Smart FW, Van Meter CH, Claycomb WC: Cardiomyocyte transplantation in a porcine myocardial infarction model. *Cell Transplant* 1998;7:239-246.
- Ghostine S, Carrion C, Souza LC, Richard P, Bruneval P, Vilquin JT, Pouzet B, Schwartz K, Menasche P, Hagege AA: Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction. *Circulation* 2002;106:II131-136.
- Tomita S, Li RK, Weisel RD, Mickle DA, Kim EJ, Sakai T, Jia ZQ: Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* 1999;100:II247-256.
- Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P: Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;410:701-705.
- Li RK, Jia ZQ, Weisel RD, Merante F, Mickle DA: Smooth muscle cell transplantation into myocardial scar tissue improves heart function. *J Mol Cell Cardiol* 1999;31:513-522.
- Min JY, Yang Y, Converso KL, Liu L, Huang Q, Morgan JP, Xiao YF: Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. *J Appl Physiol* 2002;92:288-296.
- Chiu RC, Zibaitis A, Kao RL: Cellular cardiomyoplasty: Myocardial regeneration with satellite cell implantation. *Ann Thorac Surg* 1995;60:12-18.
- Leor J, Prentice H, Sartorelli V, Quinones MJ, Patterson M, Kedes LK, Kloner RA: Gene transfer and cell transplant. An experimental approach to repair a 'broken heart'. *Cardiovasc Res* 1997;35:431-441.
- Leontiadis E, Manginas A, Cokkinos DV: Cardiac repair—fact or fancy? *Heart failure reviews* 2006;11:155-170.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143-147.
- Fukuda K: Reprogramming of bone marrow mesenchymal stem cells into cardiomyocytes. *C R Biol* 2002;325:1027-1038.
- Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu RC: Marrow stromal cells for cellular cardiomyoplasty: Feasibility and potential clinical advantages. *J Thorac Cardiovasc Surg* 2000;120:999-1005.
- Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F: Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998;279:1528-1530.
- Wakitani S, Saito T, Caplan AI: Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve* 1995;18:1417-1426.
- Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD: Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 2002;105:93-98.
- Mosca JD, Hendricks JK, Buyaner D, Davis-Sproul J, Chuang LC, Majumdar MK, Chopra R, Barry F, Murphy M, Thiede MA, Junker U, Rigg RJ, Forestell SP, Bohnlein E, Storb R, Sandmaier BM: Mesenchymal stem cells as vehicles for gene delivery. *Clin Orthop* 2000;S71-90.
- Kogler G, Sensken S, Airey JA, Trapp T, Muschen M, Feldhahn N, Liedtke S, Sorg RV, Fischer J, Rosenbaum C, Greschat S, Knipper A, Bender J, Degistirici O, Gao J, Caplan AI, Colletti EJ, Almeida-Porada G, Muller HW, Zanjani E, Wernet P: A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J Exp Med* 2004;200:123-135.



- 19 Romanov YA, Svintsitskaya VA, Smirnov VN: Searching for alternative sources of postnatal human mesenchymal stem cells: Candidate msc-like cells from umbilical cord. *Stem Cells* 2003;21:105-110.
- 20 Covas DT, Siufi JLC, Silva ARL, Orellana MD: Isolation and culture of umbilical vein mesenchymal stem cells. *Braz J Med Biol Res* 2003;36:1179-1183.
- 21 Airey JA, Almeida-Porada G, Colletti EJ, Porada CD, Chamberlain J, Movsesian M, Sutko JL, Zanjani ED: Human mesenchymal stem cells form purkinje fibers in fetal sheep heart. *Circulation* 2004;109:1401-1407.
- 22 Deten A, Volz HC, Briest W, Zimmer HG: Cardiac cytokine expression is upregulated in the acute phase after myocardial infarction. *Experimental studies in rats. Cardiovasc Res* 2002;55:329-340.
- 23 Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I: The ishage guidelines for cd34+ cell determination by flow cytometry. *International society of hematotherapy and graft engineering. J Hematother* 1996;5:213-226.
- 24 Zimmer HG, Gerdes AM, Lortet S, Mall G: Changes in heart function and cardiac cell size in rats with chronic myocardial infarction. *J Mol Cell Cardiol* 1990;22:1231-1243.
- 25 Patten RD, Aronovitz MJ, Deras-Mejia L, Pandian NG, Hanak GG, Smith JJ, Mendelsohn ME, Konstam MA: Ventricular remodeling in a mouse model of myocardial infarction. *Am J Physiol* 1998;274:H1812-1820.
- 26 Regula KM, Rzeszutek MJ, Baetz D, Seneviratne C, Kirshenbaum LA: Therapeutic opportunities for cell cycle re-entry and cardiac regeneration. *Cardiovasc Res* 2004;64:395-401.
- 27 Deten A, Holzl A, Leicht M, Barth W, Zimmer HG: Changes in extracellular matrix and in transforming growth factor beta isoforms after coronary artery ligation in rats. *J Mol Cell Cardiol* 2001;33:1191-1207.
- 28 Kadivar M, Khatami S, Mortazavi Y, Shokrgozar MA, Taghikhani M, Soleimani M: In vitro cardiomyogenic potential of human umbilical vein-derived mesenchymal stem cells. *Biochem Biophys Res Commun* 2006;340:639-647.
- 29 Boltze J, Kowalski I, Forschler A, Schmidt U, Wagner D, Lobsien D, Emmrich J, Egger D, Kamprad M, Blunk J, Emmrich F: The stairway: A novel behavioral test detecting sensorimotor stroke deficits in rats. *Artif Organs* 2006;30:756-763.
- 30 Chen SH, Chang FM, Tsai YC, Huang KF, Lin CL, Lin MT: Infusion of human umbilical cord blood cells protect against cerebral ischemia and damage during heat-stroke in the rat. *Exp Neurol* 2006;199:67-76.
- 31 Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC: Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* 2004;428:668-673.
- 32 Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JJ, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ: Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 2004;428:664-668.
- 33 Deten A, Volz HC, Clamors S, Leiblein S, Briest W, Marx G, Zimmer HG: Hematopoietic stem cells do not repair the infarcted mouse heart. *Cardiovasc Res* 2005;65:52-63.
- 34 Mollmann H, Nef HM, Kostin S, von Kalle C, Pilz I, Weber M, Schaper J, Hamm CW, Elsasser A: Bone marrow-derived cells contribute to infarct remodeling. *Cardiovasc Res* 2006;71:661-671.
- 35 Nygren JM, Jovinge S, Breitbach M, Sawen P, Roll W, Hescheler J, Taneera J, Fleischmann BK, Jacobsen SE: Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat Med* 2004;10:494-501.
- 36 Moelker AD, Baks T, Wever KM, Spitskovsky D, Wielopolski PA, van Beusekom HM, van Geuns RJ, Wnendt S, Duncker DJ, van der Giessen WJ: Intracoronary delivery of umbilical cord blood derived unrestricted somatic stem cells is not suitable to improve lv function after myocardial infarction in swine. *J Mol Cell Cardiol* 2007;42:735-745.
- 37 Kim BO, Tian H, Prasongsukarn K, Wu J, Angoulvant D, Wnendt S, Muhs A, Spitskovsky D, Li RK: Cell transplantation improves ventricular function after a myocardial infarction: A preclinical study of human unrestricted somatic stem cells in a porcine model. *Circulation* 2005;112:196-104.
- 38 Leor J, Guetta E, Feinberg MS, Galski H, Bar I, Holbova R, Miller L, Zarin P, Castel D, Barbash IM, Nagler A: Human umbilical cord blood-derived cd133+ cells enhance function and repair of the infarcted myocardium. *Stem Cells* 2006;24:772-780.
- 39 Ma N, Stamm C, Kaminski A, Li W, Kleine HD, Muller-Hilke B, Zhang L, Ladilov Y, Egger D, Steinhoff G: Human cord blood cells induce angiogenesis following myocardial infarction in nod/scid-mice. *Cardiovasc Res* 2005;66:45-54.
- 40 Hirata Y, Sata M, Motomura N, Takanashi M, Suematsu Y, Ono M, Takamoto S: Human umbilical cord blood cells improve cardiac function after myocardial infarction. *Biochem Biophys Res Commun* 2005;327:609-614.
- 41 Henning RJ, Abu-Ali H, Balis JU, Morgan MB, Willing AE, Sanberg PR: Human umbilical cord blood mononuclear cells for the treatment of acute myocardial infarction. *Cell Transplant* 2004;13:729-739.
- 42 Rocha V, Gluckman E: Clinical use of umbilical cord blood hematopoietic stem cells. *Biol Blood Marrow Transplant* 2006;12:34-41.
- 43 O'Neill TJT, Wamhoff BR, Owens GK, Skalak TC: Mobilization of bone marrow-derived cells enhances the angiogenic response to hypoxia without transdifferentiation into endothelial cells. *Circ Res* 2005;97:1027-1035.
- 44 Wollert KC, Drexler H: Clinical applications of stem cells for the heart. *Circ Res* 2005;96:151-163.
- 45 Caplan AI, Dennis JE: Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 2006;98:1076-1084.
- 46 Willems IE, Havenith MG, De Mey JG, Daemen MJ: The alpha-smooth muscle actin-positive cells in healing human myocardial scars. *Am J Pathol* 1994;145:868-875.
- 47 Sun Y, Weber KT: Infarct scar: A dynamic tissue. *Cardiovasc Res* 2000;46:250-256.
- 48 Gabbiani G: Evolution and clinical implications of the myofibroblast concept. *Cardiovasc Res* 1998;38:545-548.
- 49 Frangogiannis NG, Shimoni S, Chang SM, Ren G, Dewald O, Gersch C, Shan K, Aggeli C, Reardon M, Letsou GV, Espada R, Ramchandani M, Entman ML, Zoghbi WA: Active interstitial remodeling: An important process in the hibernating human myocardium. *J Am Coll Cardiol* 2002;39:1468-1474.
- 50 Yano T, Miura T, Ikeda Y, Matsuda E, Saito K, Miki T, Kobayashi H, Nishino Y, Ohtani S, Shimamoto K: Intracardiac fibroblasts, but not bone marrow derived cells, are the origin of myofibroblasts in myocardial infarct repair. *Cardiovasc Pathol* 2005;14:241-246.